19th INTERNATIONAL CONGRESS ON ACOUSTICS
MADRID, 2-7 SEPTEMBER 2007

EXPERIMENTAL INVESTIGATION OF THERMAL RESPONSE OF THE BACKSCATTER FROM ULTRASOUND CONTRAST AGENT SONOVUE™ BY MEANS OF A COMMERCIAL CLINICAL ULTRASOUND EQUIPMENT.

PACS: 43.40.Fz

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ABSTRACT

Extraordinary progresses have been made in the diagnostic potential of ultrasonography in recent years, due to the combination of ultrasound contrast agents and contrast specific imaging techniques. Dedicated ultrasonography using low acoustic energy emission (Mechanical Index <0,1) applied to contrast microbubbles, the so-called second generation, produces reduced intravascular microbubble destruction, allowing prolonged in vivo circulation time of the contrast agents and Real-Time continuous scanning. The object of this article is to measure the frequency response of SonoVue™ at various temperature, ranging from 37 to 43 °C. This range corresponds to the temperature variations imposed to tissues during hyperthermia treatments in oncological applications. The method is based on the spectral analysis in vitro of the backscattered Radio Frequency signals acquired from commercial ultrasound scanning equipment, modified ad hoc for this purpose, with a convex array transducer. This feature allowed us to analyze in the frequency domain the backscattered echoes coming from a region of interest (ROI) of relatively large size.

1. INTRODUCTION

Ultrasound Contrast Agents (UCAs) play a major role in diagnostic ultrasound and are becoming increasingly important in therapeutic applications. Contrast-enhanced ultrasonography is a widely available and relatively inexpensive method with a high potential for tissue perfusion measurements. UCAs offer a great potential in imaging microvascular perfusion by enabling improvements in the sensitivity of the ultrasound system for detection of the slow moving blood in the smallest vessels [1]. Microbubbles typically consist of an inert gas encapsulated within a protein, lipid or polymer shell for stabilisation. Their mean diameters are roughly 5 μm so that they are able to traverse the entire capillary network after injection. Due to the large differences in compressibility (up to a factor 10.000) between the encapsulated gas microbubbles and blood, they are extremely effective in blood echo enhancing. It should be noted that, although the encapsulating shell stabilises the microbubbles by increasing their lifetime in the blood pool, its damping effect influences their acoustic properties [2]. The recent development of UCAs may open such an opportunity and permit to visualize on-line perfusion of cardiac muscle and other organs. To interpret the ultrasound scattering profiles, it is necessary to understand the transport mechanism of microbubbles in the microcirculation, specifically their transport in blood stream, the attachment to blood cells and endothelium, as well as possible leakage across the microvascular wall. Each of these events provides an opportunity to monitor with ultrasound not only the microvascular blood flow but also to detect inflammatory sites in the circulation. Around 20 percent of the heart examinations (echocardiograms) do not provide images of adequate quality to allow the visualization of the inner border of the heart (endocardial border) for accurate diagnosis of ventricular dysfunction. The development of UCAs that highlights the endocardial border has made contrast echocardiography a clinical reality. When imaging solid organs such as the liver, abnormal areas, such as a blood clot after trauma or a tumor are not visible in nearly 40 to 50 percent of cases. The ability to see blood flowing into the liver as the microbubbles percolate through its vascular space, and then when all the vascular spaces are filled, allows the physicians to recognize the specific observed pathology. Contrast echosonography is thus expected to contribute substantially to the current global emphasis of achieving earlier, more accurate, more cost effective diagnoses. When used in conjunction with an UCA, traditional B-mode suffers from a number of drawbacks, the main one being the high baseline echogenicity of most tissues, which prevents specific detection of the agent. Extensive research activity in UCAs has triggered the rapid development of novel contrast-specific imaging modes by equipment manufacturers, taking particular advantage from the non-linear properties of bubbles. Because they are tuned to the specific detection of microbubbles, those imaging modes allow enhanced contrast between the vessels that contain the UCAs and the surrounding tissues. This is particularly important for the myocardium, a strongly echogenic and moderately vascularized muscle, in which small numbers of contrast microbubbles cannot be detected reliably by conventional B-mode. Harmonic B-mode, phase or pulse inversion, power modulation, coherent contrast imaging, and recently power pulse inversion are among the new imaging modes that have considerably improved the detection of UCAs. Many of these novel imaging modes give outstanding results at low mechanical index (MI<0,3). At low MI, tissue echoes can essentially be suppressed (i.e. they appear black on the image), whereas strong echoes are generated by contrast-containing vessels. Furthermore, the SonoVue™ microbubbles can be continuously imaged without being destroyed, allowing them to reach the microcirculation and its large blood volume and low blood velocity. With contrast-specific imaging modes, tissues perfused with UCA containing blood become highly visible and perfusion defects emerge as black areas in Real-Time examinations. SonoVue™ is particularly effective at low MI (<0,1 or less) and is the first ultrasound contrast agent which allows repeated continuous examination of the liver in real time with conventional B-mode sonography for the detection of hepatic metastases. The UCAs, proposed originally for visualization and diagnostic purposes, have been recently [3] suggested as efficient enhancers of ultrasonic power deposition in tissue, moreover might have beneficial impact in therapeutic applications such as targeted hyperthermia-based or ablation treatments. Introduction of gas microbubbles into the tissue to be treated can improve the effectiveness of current treatments by limiting the temperature rise to the treated site and minimizing the damage to the surrounding healthy tissues.

The current study was designed to investigate experimentally the thermal behaviour of SonoVue™ in the 37 °C to 43 °C temperature range with steps of 1°C. The measurements were performed through the use of a commercial imaging system with a pulse inversion imaging method after injection of SonoVue™. The use of a commercial scanner will lead to an improvement with respect to *in vitro* set-up based on single element transducers [4].

2. MATERIALS AND METHODS

2.1 Microbubbles

The contrast agent used in this study was SonoVue™ [5] (Bracco Research SA, Geneva, Switzerland), constituted by 25 mg of lyophilized dry powder in an atmosphere of perfluorochemical SF_6 gas contained in a glass vial with elastomeric closure. The lyophilysate is composed of a combination of pharmaceutical grade polyethylene glycol 4000 and phospholipids (distearoylphosphatidylcholine and dipalmitoylphosphatidylglycerol). The agent is prepared before use by injecting with 5 ml of 0,9 percent saline solution through the septum to the content of the vial. A suspension of microbubbles (2,5 μm mean diameter in the range from 0,7 to 10 μm; $1\div5\cdot10^8$ microbubbles/ml) stabilized by a phospholipidic monolayer is reconstituted. The vial is then shaken vigorously for 20 s after which the desired volume of the dispersion can be drawn. The total volume of SF_6 gas in the bubbles is approximately 8 μl/ml of the reconstituted suspension. In the present study the dilution was 1:2000 for the native suspension, corresponding to approximately $1{,}5\cdot10^5$ microbubbles/ml. This concentration was selected because it corresponds to a optimal dose, such reported in [6].

2.2 Acoustic Response Modeling of SonoVue™

To predict the behavior of microbubbles, several theoretical models have been proposed. First of all, in 1917, Lord Rayleigh [7] studied cavitation bubbles around ship propellers. Minnaert [8] in 1933 performed a theoretical study of the sound emission of bubbles.

Combined with some experiments, he explained the characteristic resonance frequency. In the early 1950's Plesset and Nolting and Neppiras introduced more sophisticated models for oscillating bubbles, followed by refinements in the late 1950's (Keller, Gilmore, Herring, Trilling) and the 1980's by Keller and Miksis and Prosperetti. Encapsulated microbubbles were first modeled by [9] in 1992 and [10] in 1993, incorporating experimentally determined elasticity and friction parameters into the Rayleigh-Plesset model. Church [11] used linear visco-elastic constitutive equations to describe the shell. A recent addition to the list of models for contrast agent dynamics is given in [12]. The model is based on the modified Herring equation. The resonance frequency of coated bubbles can be derived numerically for small amplitude oscillations, $r(t)=r_0(1+\varepsilon(t))$ with $\varepsilon(t)$ a small disturbance on the radius. An analytical expression is derived:

$$f_R = \frac{1}{2\pi}\cdot\sqrt{\frac{3\gamma p_0}{\rho r^2}+\frac{2\sigma\cdot(3\gamma-1)}{\rho r^3}+\frac{6\chi\cdot(\gamma-1)}{\rho r^3}} \quad \text{(Eq. 1)}$$

where p_0 represent the ambient hydrostatic pressure = 101.325 kPa, ρ is the density of surrounding fluid, χ is the shell stiffness = 0,26 N/m, σ is the surface tension at the outer interfaces between the shell and the liquid = 0,051 N/m and γ is the polytropic gas exponent = 1,07. The first two terms are the same found for the expression for the resonance frequency of free gas bubbles. The additional third term accounts for the shell effects.

2.3 The experimental set-up and acoustic measurements

The experimental set-up showed in Figure 1 includes the following building blocks:

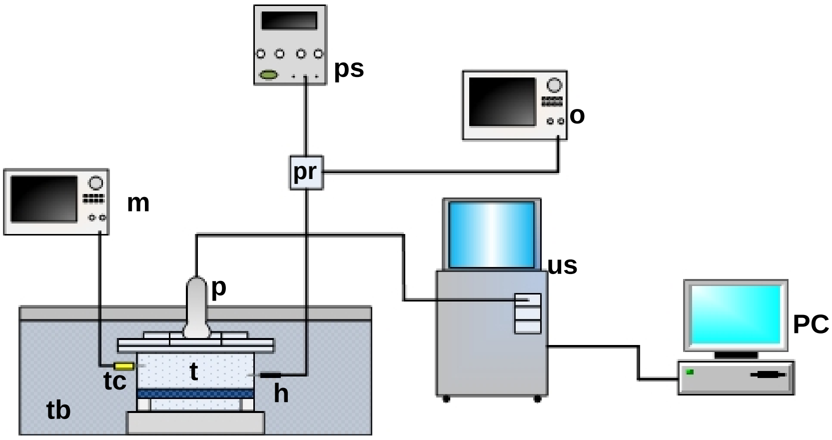

Figure 1 - The experimental set-up composed by: **p**, probe; **us**, ultrasound scanner; **tb**, thermoregulated bath; **tc**, thermocouple; **t**, tank; **h**, needle hydrophone; **m**, multimeter; **o**, oscilloscope; **ps**, hydrophone power supply; **pr**, hydrophone preamplifier. Suspensions of SonoVue™ was insonified using an commercial imaging system, which was controlled by a PC. The RF data were stored in the PC for processing and analysis.

The set-up and experimental protocol

The tank used in the experiments was filled with roughly 1350 ml of distilled water. Its size was 100 mm high x 160 mm length x 120 mm wide. The distance of the scanning surface of the probe from the bottom of the tank was located at 68 mm, which was the length of the focal zone. The pulse amplitude was measured inside the tank at 50 mm with a needle hydrophone of 0,5 mm diameter disc (Precision Acoustics Ltd., Hampton Farm Business Park, Higher Bockhampton, Dorchester, Dorset DT2 8QH, UK). The peak negative pressure was 28 ± 3 kPa, which corresponds to MI = 0,112 at 1,79 MHz. A hole of 80 mm length x 40 mm wide was drilled at the top of the tank and a cellophane film was fixed to provide an acoustic window. Two measurements were carried out: first a reference measurement with the distilled water without microbubbles, then a measurement with the SonoVue™ were introduced into the tank using a syringe (1000 Series GASTIGHT™ TLL Syringes, Hamilton Company, Reno, NV) with volume of 675 µl mixed with the previous distilled water. After the introduction, the suspension was stirred for 10 s and data successively acquired. In the first measurement the ultrasound beam was reflected from a polished aluminum block positioned at the bottom of the tank, which is taken as a close approximation to a perfect reflector, whose intensity reflection coefficient was calculated equal to 0,84. In the second measurement a polyurethane rubber absorber [13] of 14 mm thickness (National Physical Laboratory, Teddington, Middlesex, UK, TW11 0LW) was positioned on the wall of the tank opposite the transducer to minimize the influence of acoustic reflection within the water tank. The absorber exhibits a dependence with temperature, whose temperature coefficient of the transmission loss at 1 MHz is 1 $dB \cdot °C^{-1}$, resulting in a transmission loss of approximately 50 $dB \cdot cm^{-1}$ at 40 °C. Measurements are carried out by thermostating the water tank in a thermoregulated bath (DIGI-100, Analitica De Mori, Milan, Italy) filled with distilled water. The temperature inside the tank is monitored with a thermocouple K fixed at 35 mm from the transducer. After thermalization the probe is insonified at a set of temperatures, in the range 37-43 °C with steps of 1°C. This test measurements took approximately 50 min.

The scanner and the acquisition data

The suspensions of SonoVue™ were insonified using an TECHNOS MPX scanner (ESAOTE Spa, Italy), which was connected to a PC through its SCSI port. A non linear imaging modality, (contrast tuned imaging CnTi) was performed using a broadband convex array transducer that utilizes 3,5 MHz for fundamental imaging and 1,79 MHz for CnTi. This latter technique was adopted and thus the bubbles were insonified at 1,79 MHz. A major advance for sonographic examinations with contrast agents is the development of pulse inversion imaging [14]. In this technique two pulses are sent in succession into the body. The second pulse is a mirror image of the first. The scanner detects the echo from these two successive pulses and forms their sum. For linear tissues, the sum of two inverted pulses is zero. On the other hand, for an echo with nonlinear components, such as that from a bubble, the echoes produced from these two pulses will not be mirror images of each other because of the asymmetric behavior of the bubble radius with time. The result is that the sum of these two echoes is not zero. Thus, a signal is detected from a bubble but not from tissue. Mathematical analysis shows that this summed echo contains the nonlinear “even” harmonic components of the signal, including the second harmonic. Bubbles undergo stable, nonlinear oscillation, emitting three backscattered signal components: 1) the fundamental and 2) the harmonics with respect to the frequency content of the pulse emitted by the probe and 3) the resonance frequency of the microbubbles depending on their diameter and other variables such as the temperature. The first two can be considered as *forced responses*, while the third is the *free oscillation* of the microbubbles both driven by the wide bandwidth ultrasonic pulse. A proprietary data acquisition module manufactured by ESAOTE was used to acquire the Radio Frequency (RF) signals from the scanner. Each frame of data consisted of 708 scan lines for 2208 samples of RF data. A maximum of 30 frames were acquired during real-time scanning at a frame rate of 9 Hz. A complete acquisition to PC, for each frame, took on the order of 4 min. The RF data are analysed off – line with a dedicated software (MatLab®, The MathWorks Inc. Natick, MA, USA).

2.4 RF analysis technique

In the present work the collected data were accomplished in a multiple step process. The analysis was based on sum of columns even and odd of the RF data. Even columns are constituted by the scattered signal when no inversion was applied to the transmitted pulse, while odd columns come from the inverted (180 degrees) pulse. For our purposes, the sum of adjacent columns eliminate the driving frequency of the convex array and thus the “common mode” signal generated by the forced response of the bubbles driven by the ultrasonic pulse. The new RF data were in 353 lines x 2208 samples format, moreover the echo signals were time gated using a 64 µs (1600 samples) gate. The SonoVue™ power spectral density of the gated portions of the 801 samples were computed from the average of 291 lines. The mean power spectrum was then normalized by the power spectrum of the reference waveform obtained from the specular echo from the aluminium reflector to compensate the electromechanical conversion factor of the transducer and the instrumentation. This power spectrum was computed from the scan line with the maximum amplitude of the echo signals. This yielded the average power spectrum of the backscattered transfer function. This procedure was repeated for each temperature to determine the peak associated with the first resonance frequency of the microbubbles [15].

3. RESULTS AND DISCUSSION

The average diameter of the SonoVue™ varies as a function of temperature and in Figure 2 the mean value and standard deviation of the microbubble diameters measured in the 37- 43 °C temperature range, is reported from [16]. Therefore, referring to eq. (1) the resonance frequency was calculated at different diameters represented in Table 1 and compared with the experimental values.

Table 1 – Theoretical and experimental values of the resonance frequency at various temperatures.

Temperature (°C)	Theoretical mean values of resonance frequency (MHz)	Experimental mean values of microbubbles first resonance frequency (MHz)
37	7,04	2,60
38	N.A.	3,78
39	7,27	3,73
40	2,88	2,56
41	5,51	N.A.
42	7,26	2,69
43	10,75	3,67

The Figure 2 shows the experimental thermal response and the predicted values with the modified Herring model of the resonance frequency versus the temperature.

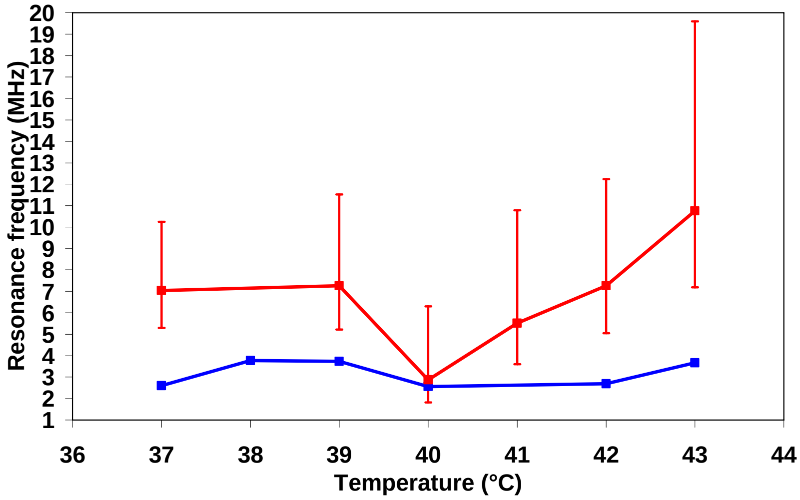

Figure 2 - Resonance frequency of the microbubbles vs. temperature. The red squares are the theoretical values for SonoVue™ predicted by eq. (1) using the mean and standard deviation of the microbubbles diameter taken from ref. [16]. The blue squares are the measured resonance frequencies.

As it can be seen from the figure, the experimental thermal behavior is in reasonable agreement with the theoretical model. The experimentally measured frequency is generally lower than the calculated one. This may be due to the fact that eq. 1 and those proposed by [15] do not consider the variation with temperature of several quantities in particular, the elastic shear modulus of the shell decreases vs. temperature, and so the resonance frequency. These considerations suggest that further work is needed to explore the multiple link between the cited parameters and the frequency response of the bubbles. In the current study a different experimental set-up was presented and evaluated compared to others used for *in vitro* experiments [18].

Acknowledgement

This work was partially supported by Italian Ministry of Education, University and Research under grant No. RBAU01HHMC_001. The Authors are grateful to dr. A. Ceraolo of ESAOTE Spa for providing the modified ad hoc ultrasound scanner.

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